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Abstract

Elastic scattering spectroscopy (ESS) is a technique for interrogating tissue with short pulses of white light and undertaking an immediate spectral analysis of light scattered back to obtain diagnostic information. The program aims to assess the value of ESS for detecting cancer in breast tissue and axillary lymph nodes. There are 4 objectives.

1. Develop new algorithms for analyzing existing spectra matched to conventional biopsies (data pairs) in cancer containing breast tissue. Best results give 95% sensitivity and 71% specificity for detecting cancer
2. Collect and analyze additional data pairs from axillary sentinel lymph nodes. Results are weaker on partially metastatic nodes due to sampling errors, which are being addressed.
3. Assess whether ESS can be used to detect aneuploidy, as a prognostic feature. We have established a laboratory reference technique to measure aneuploidy, and begun data collection, but need more data prior to attempting correlation with ESS spectra.
4. Assess whether ESS can be used to diagnose lesions seen at ductoscopy (nipple endoscopy). We now have data pairs from 6 breasts examined ex vivo after mastectomy.

Encouraging progress has been made with each objective, but much more data are required before firm conclusions can be drawn.

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INTRODUCTION

Breast cancer remains the most common malignancy affecting women within the western world, and until recently accounted for the highest number of cancer deaths in women. Epidemiological data shows an ongoing rise in the incidence within the western world, though within recent years there have been modest improvements in mortality.

Greater public awareness of breast cancer as a result of effective education campaigns is resulting in earlier presentation of breast cancer. Early diagnosis of breast cancer prior to the development of metastases results in a greater chance of cure.

The focus of this research program is the development of an optical technique called elastic scattering spectroscopy (ESS) for detection, assessing the extent of spread (staging) and prognostication of early breast cancer.

Numerous optical methods of diagnosis are under investigation worldwide(1). The key advantages of optical techniques such as ESS are:
- Speed of diagnosis: Since computer based algorithms can be developed for spectral analysis, a near instantaneous result is achievable
- No highly trained operator is required for measurements or analysis
- Safety: Avoids the use of ionizing radiation for diagnosis
- No tissue loss: Analysis by optical measures is non-destructive to tissue; hence tissue can be re-analyzed conventionally to confirm accuracy

Elastic scattering spectroscopy has further advantages, which include:
- Low cost equipment
- Portability: The system can be easily transported and set up within the clinic or operating room
- Diagnostic information obtained from deeper layers: The system uses information gathered down to approximately 0.3mm in depth from the tissue surface.

Theoretical Background to Elastic Scattering Spectroscopy

The principle of elastic scattering spectroscopy (ESS) is that tissue is interrogated by short pulses of white light, delivered by a thin optical fiber just touching the tissue surface. The light scattered by the tissue is collected by a second fiber immediately adjacent to the first (center-centre 0.35mm) and the diagnostic information obtained by statistical analysis of the spectrum of the scattered light.

When performed using an appropriate optical geometry, the spectrum is sensitive to the sizes, indices of refraction and structures of the sub-cellular components (eg nucleus, nucleolus and mitochondria) that change with malignant transformation(2;3). The measured ESS spectra relates to the wavelength-dependence and angular-probability of the scattering efficiency of tissue micro-components (as well as to absorption bands) based on the fact that many tissue

pathologies and most cancers undergo such morphological changes at the cellular and sub-cellular level.

Consequently this approach generates spectral signatures, which reflect the tissue parameters that pathologists address, such as the size and shape of nuclei and organelles, the nucleo-cytoplasmic ratio and chromatin density. Both Mie theory and finite-difference time domain methods have been employed successfully to model spectral changes resulting from malignant transformation. Multivariate statistical analysis can be used to recognize patterns within the spectra, which can then be used to discriminate between spectra from malignant and benign tissue once appropriate diagnostic algorithms have been developed.

Elastic Scattering Spectroscopy Equipment

The ESS instrumentation consists of a pulsed xenon arc lamp, a spectrometer, an optical probe and a computer to control the various components and record the spectra. The arc lamp, spectrometer and power supply are housed in a briefcase size unit to which the laptop computer is connected. ESS involves directing short pulses of white light (320-920nm) from a pulsed xenon arc lamp (Perkin Elmer, Inc.) through a flexible optical fiber touching the tissue to be interrogated. Ultraviolet B (280-315nm) and ultraviolet C (100-280nm) light is filtered out to avoid any potential risk to patients.

A collection fiber, with a fixed separation distance of ~350 μm from the first, collects light scattered from the upper layers of the tissue and propagates it to the spectrometer (S2000 Ocean Optics) which outputs the spectrum to the laptop computer for recording and further analysis.

Figure 1 shows a schematic diagram of the system, and figure 3 shows a depiction of the optical geometry employed within the system. Typically, the whole fiber assembly measures 1.5 mm and the distal end may be housed in a rigid stainless steel casing for easy handling and sterilization. The collection and recording of a single spectrum takes less than a quarter of a second

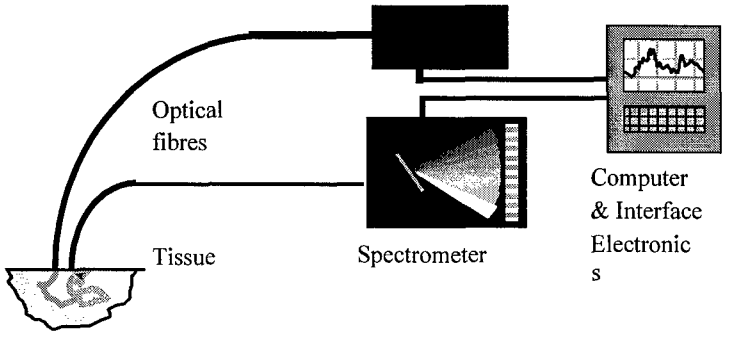

Figure 1. Schematic diagram of Elastic Scattering Spectroscopy (ESS) system.

Figure 2. Photograph of ESS Optical Biopsy System

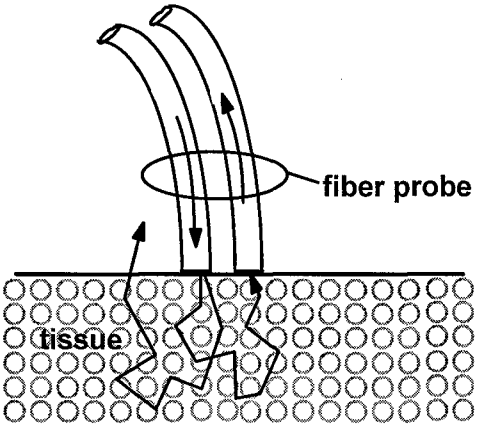

Figure 3. Depiction of the optical geometry used in the ESS method. Fiber tips are in optical contact with the tissue surface. With a fiber separation of ≤350 microns (center-to-center) only light that has scattered elastically a small number of times, and at large angle within a shallow layer is collected.

Before any spectra are taken, a white reference spectrum is recorded. The reference spectrum establishes the system response by recording the diffuse reflectance from a flat surface of Spectralon™ (Labsphere Inc.). Spectralon has a spectrally flat reflectance between 250 and 1000 nm. The reference spectrum allows variation in the light source, spectrometer, fiber transmission and fiber coupling to be accounted for. Each recorded ESS spectrum from tissue is divided by this reference spectrum to give a system-independent measurement for the site being investigated.

BODY

REGULATORY ASPECTS

The program has received ethics committee (Institutional Review Board) approval from the Joint University College London and University College London Hospitals Ethics committee.

The program was submitted as 2 separate ethics applications:

1. Optical biopsy utilizing elastic scattering spectroscopy for early diagnosis, staging and prognostication in breast cancer (Short title : Optical Biopsy for Diagnosis, Staging & Prognosis in Breast Cancer). A favorable ethical opinion was given on the 17th of November 2004, under reference number 04/Q0502/83. Trust research and development approval for this study was granted on the 21st January 2005 under reference number 04/0109.

2. Ex-vivo ductoscopy with elastic scattering spectroscopy for the diagnosis of ductoscopic abnormalities and light dosimetry studies (Short title: Light for diagnosis and treatment of ductoscopic abnormalities). A favorable ethical opinion was given on the 19th of November 2004, under reference number 04/Q0502/40. Trust research and development approval for this study was granted on the 21st January 2005 under reference number 04/0209. Note that a separate sub-protocol of light dosimetry experiments unrelated to this particular program was included within this ethics application.

A further ethics application will need to be submitted in the future for the in-vivo ductoscopy work. It is planned to submit this once we have sufficient data from the ex-vivo study to support this application.

An independent initial Human Subjects Protection Review has been conducted by the USAMRMC Office of Research Protections, on the 18th of March 2005, under reference numbers A-12727-1 and A-12727-2. The protocols have been deemed "Greater than minimal risk" and some amendments to the documentation were requested; these have been submitted and will be considered by the Human Subjects Research Review Board on the 13th of July 2005.

There have been considerable, but unavoidable delays in completing the formalities for approval of the clinical protocols. Final approval has not yet been granted by the USAMRMC Office of Research Protections. The main reason for the severe delay was that just before the IRB application was prepared, the entire system of ethics committee approval throughout the European Union was changed to bring in uniformity throughout all 25 member countries of the Union. The new system was far more complex than the previous one. Much of the delay was because those administering the new system did not have the necessary guidelines on how to proceed! The new system is now settling in, but further delays occurred due to the backlog of applications that built up during the transition period.

As final approval has not yet been granted by the USAMRMC Office of Research Protections, we have not been able to use any of the grant money for aspects of the studies that required USAMRMC approval. However, over the last year, we have been able to support most of Dr Chicken's salary from other sources. As the protocols related to this study were fully approved by our local ethics committee and hospital research and development office in January 2005, this made it possible for us to proceed with some of the clinical studies since then. Although much of this work was not supported by the present grant, the results are presented here as we propose to continue this work on the Army grant as soon as the USAMRMC Office of Research Protections gives final approval.

Our original grant application was for a period of 2 years. When we learnt that the amount of the award was to be reduced, our revised statement of work limited the research period to 18 months. As we have been unable to proceed with clinical studies on the grant so far because of the IRB delays and we have been able to support Dr Chicken from other sources to fill this gap, we should now like to request that the grant period is restored to the original duration of 2 years. This would give a finishing date of 31st May 2006. We would keep to the original budget.

Personnel:

Dr Kristie Johnson (physicist) resigned to take up a position in Sydney, Australia, and has been replaced by Dr Benjamin Clark (physicist). Apart from this, all personnel remain as identified in the original proposal.

Aims and Rationale of Research

This research program has 4 primary aims:

1. To refine algorithms and develop new techniques for analysis of ESS spectra

2. To develop elastic scattering spectroscopy for the immediate detection of metastatic cancer in axillary sentinel lymph nodes. The sentinel node is any node with direct lymphatic drainage from a primary tumour and is by definition the first node to be involved when lymphatic metastases occur. Sentinel node biopsy is an accurate procedure for lymphatic staging in breast cancer that avoids the morbidity of full axillary lymph node dissection in node negative patients. The rationale for this part of the study is that patients with sentinel node metastases require axillary lymph node dissection (clearance) for regional control of the disease. Unless sentinel node metastases can be diagnosed at the time of sentinel node biopsy, a second operation is required for axillary lymph node dissection, with its associated psychological and economic costs and consequent delay in commencement of adjuvant therapies. Existing means for intraoperative detection of sentinel node metastases (frozen section, touch imprint cytology) rely on the expert opinion of a pathologist and typically take 30 minutes for a result.

3. To develop elastic scattering spectroscopy for the detection of aneuploidy. The rationale for this is that ploidy shows promise as an independent prognostic marker in breast cancer. Laboratory determination of ploidy is however time consuming and requires expensive equipment. ESS may offer a rapid and simple alternative.

4. To develop elastic scattering spectroscopy for diagnosis of pathology visualized at ductoscopy. Ductoscopes are small caliber fibre-optic endoscopes which are introduced into the ductal system through the nipple. 80% of breast cancers are ductal in origin. Ductoscopy may be used to detect very small tumors (as small as 0.2mm) or pre-invasive changes such as ductal carcinoma in situ (DCIS). A role for ductoscopy has been suggested in determining appropriate margins for excision of tumors as well as for the assessment of blood stained nipple discharge, which may be a presenting symptom of breast cancer. The rationale for this part of the study is that the small caliber of ductoscopes prevents taking histological samples for confirmation of the diagnosis. Optical biopsy may offer the ability for instant diagnosis, and in the longer term, potentially, immediate therapy.

Objective 1. New techniques for analyzing data pairs from our existing database on breast tissue.

Current Method for Analysis of Spectra

Currently, the analysis consists of:

1. Cropping spectra to remove uninformative regions (regions at the end of the spectrum where the signal: noise ratio is low).
2. Combining the spectra to reduce the number of measurements.
3. Smoothing by Savisky-Golay method
4. Removal of spectra negatively saturated at the hemoglobin peaks
5. Normalisation/Standardisation of spectra using the standard normal variate method. This consists of subtracting the mean intensity and dividing by the standard deviation of the spectrum, giving spectra with a mean of zero and a variance of ± 1.
6. Data compression using Principal Component Analysis (PCA)
7. Discriminant analysis using Linear (LDA) or Quadratic Discriminant Analysis (QDA), depending on which gives the better discrimination. The number of principle components used is determined by either a forward stepwise method, or a pragmatic search of the possible combinations of principle components.
8. The data is then split into a training set and test set and the final QDA or LDA analysis is run on the data and the test set results are recorded.
9. Models are validated using re-sampling techniques whereby different training/test set combinations are randomly assembled and the analysis is repeated, yielding an estimate of the behavior of the algorithm on average.
10. For each model, the tradeoff between sensitivity and specificity is visualized using a receiver-operator curve (ROC curve). This is calculated by altering the canonical scores (from an LDA or QDA). Once the relationship between sensitivity and specificity has been estimated, an appropriate cutoff point can be decided upon. The final algorithm consists of PCA loadings, and Discriminant analysis coefficients (Canonical Coefficients, or Classification Coefficients) and the cutoff canonical score.

Algorithms are run in Systat (SYSTA Software Inc), Matlab (Mathworks), or GNU R. To explore the possibilities of alternate methods of analysis, we are working on a comparative study to try to determine if there are any other analysis techniques that may improve the predictive power of our models. Possible alternative methods include partial least squares regression analysis, neural networks and support vector machines. We will apply different techniques to the ESS data and try to improve our analysis. In addition to alternative methods of analysis, we will also attempt to identify spectral features that can confound (confuse) or improve the analysis (see, for example, our attempt to remove the information associated with patients from the grid analysis in order to improve the discrimination).

Proof of Principle Study

In an earlier study we undertook a detailed analysis of large sections taken through cancers removed by mastectomy. ESS measurements were taken on an 8 by 8 grid (each pixel 2x2mm). Subsequently our histopathologist gave a detailed report, which included documenting the amount of cancer present in each pixel. By correlating the spectral measurements and the histological diagnosis, we aimed to estimate the sensitivity of the ESS device in detecting cancer. During the earlier study, the raw data from histology and ESS were obtained, but the spectral analysis was not completed. That has been done as part of the present study. In particular, we have tried to determine the smallest amount of tumor present in individual pixels that could be detected. The current analysis is based on data from five ductal cancers, yielding a total of 271 data pairs (spectral measurements and histology).

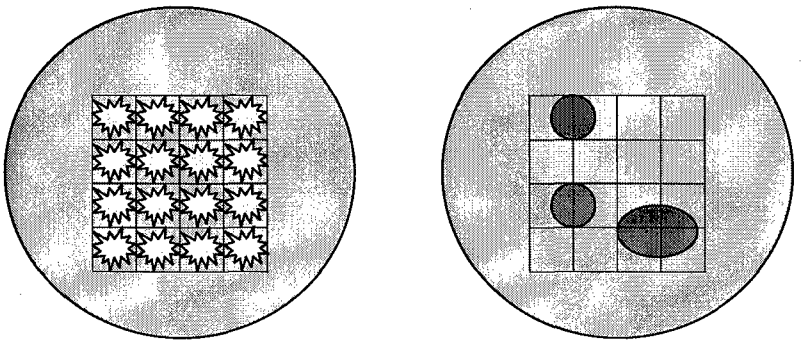

Figure 4 Schematic diagram of the grid experiment. Left: Spectral measurements were taken on a grid. Right: form of mapping of cancer areas

The grid data were analyzed with the aim of determining whether the model was capable of detecting cancer at sites where a large proportion of the tissue interrogated by the light pulses was normal. In this analysis, the model was trained on a subset of the spectra from either 100% normal (0% cancer) tissue, or 80-100% cancer. The remaining spectra were then used as a test set to assess the performance of the algorithm.

Four of the 30 principal components used in this analysis are shown plotted against each other in figure 5. A particular feature of this dataset not seen in the sentinel node study can be seen in the way that data from different sites (shown in different colors) separated out (clustered). In this context, a site is the region of breast tissue from one patient covered by the grid that contains both normal and tumor tissue. The reason that we can see this separation is due to the relatively large numbers of measurements (~ 52 per site) being taken from relatively few sites (5). In addition, the variable amount of cancer in each pixel across sites means that discrimination between normal tissue and cancer may be difficult to distinguish from discrimination between the different tumor containing sites. From the data collected so

far, it is difficult to say what physical differences contribute to this "site" effect, but it is clear that we should avoid using spectral features that may be confounded by other factors. Preliminary analysis of the parts of the spectrum that contribute to this "site" effect indicate that differences in the amount of absorbance
in the region of the Q-band of hemoglobin ~530 nm - ~620nm are mainly explained by site, and consequently differences in blood saturation and oxygenation may vary widely between tumors. There is a clear need to understand the mechanistic basis of the optical differences between normal and malignant breast tissue. We are planning phantom studies to identify the spectral signatures of major components of the tissue.

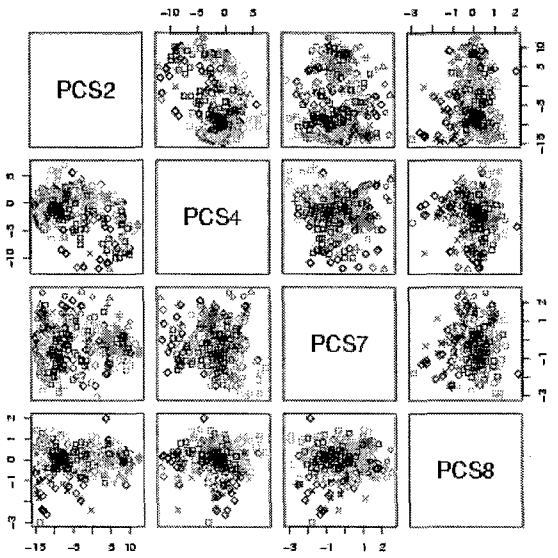

Figure 5. A pairs plot of four principal components that discriminate between sites. Each individual plot contains all of the spectra from each site (from tumor and normal areas) plotted using two principal components. On each of the plots, each site is shown in a different color. Because some of the spectral information is correlated with site of origin, spectra from a single site (i.e. from the same tumor and the immediately surrounding tissue) cluster together, leading to patches of symbols of the same color.

A naive first analysis shows that the probability of detecting tissue with small amounts of cancer in it (1-20%) is roughly 50%. Tissue with large amounts of cancer in it is classified correctly roughly 90% of the time (fig 6a). We attempted to remove parts of the spectrum in which the variance is mainly explained by site, leaving regions where the information is less confounded by the tumor of origin (i.e. with less confusing information in them). We did this by fitting a linear mixed-effects model to the data set at each point in the spectrum. At each point we calculated the amount of variance in the data explained by site, and the amount of variance left over (the *residual variance*) once this *random effect* had been estimated. By

looking at the ratio of residual: site variance, we have a measure of how much the variance (at any given point in the spectrum) is explained by site. We removed the regions where the residual: site ratio was below 1 (because this meant that most of the variance was explained by site) and re-ran the analysis with this transformed data. The overall sensitivity and specificity of the model improved slightly (fig 6b, Table 1). The regions of the spectrum removed are indicated in fig 7.

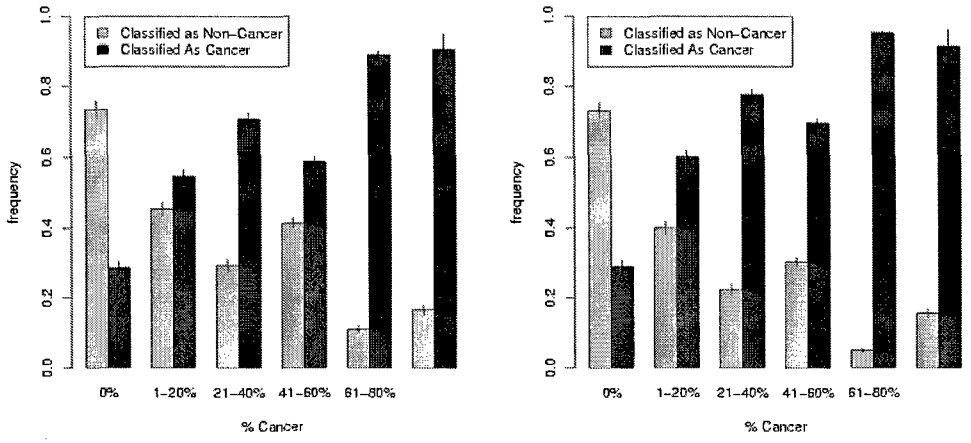

Figure 6 a) Result of the Linear Discriminant Analysis in a model with all parts of the spectra left in. b) Result of the Linear Discriminant Analysis in a model with some parts of the spectrum (shown in Figure) taken out in order to aid discrimination between tumor and normal. In comparison to the complete model (shown in 5a), the discrimination is slightly improved.

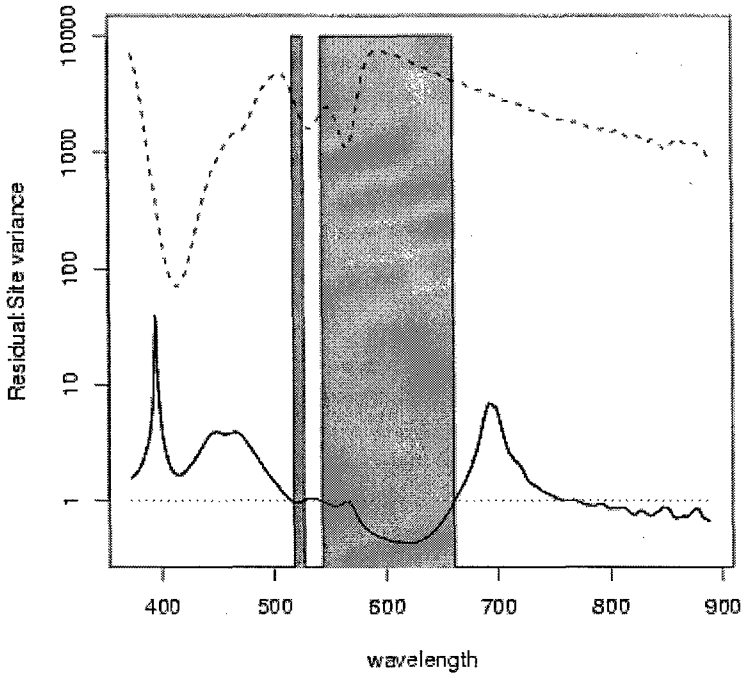

Figure 7 Line plot showing which regions contain useful information, and which regions contain information that is confounded by site. The solid line is the ratio of the residual variance: variance explained by site. The grey regions denote those parts of the spectra where most variance is explained by site of origin rather than anything else. The horizontal dotted line is where the ratio between residual variance and variance explained by site is equal to one. The region > 780 nm was left in as it improved the analysis. The dashed line is a typical spectrum (offset for clarity).

Table 1: The percentage of samples classified as cancer is an increasing function of the amount of cancer in that tissue. The small number of samples with 80-100% cancer may be reflected in the relatively low accuracy in that category (compared to those samples with 61-80% cancer, for example). When the regions shown in Figure are removed, the overall accuracy of the model improves.

	% Cancer in tissue	0%	1-20%	21-40%	41-60%	61-80%	81-100%
% Classified as Cancer	With regions left in	29	51	69	59	90	86
	With regions taken out	29	56	74	67	95	87

The results presented above have potential implications beyond the grid dataset. If some parts of the spectrum are uninformative, or are confounded with other factors such as the site of origin, identifying these regions and controlling for them or removing them would improve our ability to discriminate between normal tissue and cancer. In order to identify these regions we need enough measurements per site in order to distinguish between the effect of differences between sites and the effect of differences within a site. For the sentinel node work, we collect 16 spectra per node as part of the routine measurements, so will be able to use this data to attempt to quantify these regions, although any way of increasing the number of spectra taken from each node will benefit this analysis.

One possible area for future investigation is to identify the physical factors that contribute to this "site" effect and remove them by modeling the absorption of that factor and deducting it from the spectra, leaving the remainder of the spectrum for analysis. For example, the main area associated with the site effect lies between 540 and 660 nm, corresponding to the 580 hemoglobin peak. By modeling the hemoglobin and removing it from the spectra, we may be able to remove information from the spectra that does not help us discriminate between normal tissue and cancer.

Objective 2. Collection and analysis of new data pairs from breast tissue and axillary nodes.

During the period covered by this report we have expanded our dataset by the addition of 1376 spectra (matched with final histology results) measured from 86 sentinel and axillary nodes from 45 patients. The previous data was obtained during an earlier project funded by the USARMC (DAMD17-98-1-8343). In addition, 13 sentinel and axillary nodes have been scanned using our 2 dimensional scanner, resulting in 2600 spectra.

Currently our total dataset consists of 382 axillary nodes with 2893 spectra matched with histology. The dataset consists of:

	Number of Nodes	Number of Spectra
Normal Nodes	298	2171
Metastases detectable only with IHC	1	15
Micrometastases (<2mm diameter)	7	76
Partial Metastases (>2mm)	43	295
Total Metastatic Replacement	33	336

Mean spectra from normal and metastatic nodes are plotted below. It is re-assuring to note a distinct separation between the normal (blue line) and totally metastatic nodes (red line). The spectra from partially metastatic nodes lie intermediate to the normal and totally metastatic nodes, and this reflects that some of the spectra are taken from areas of tumor, while others are from normal areas of the node.

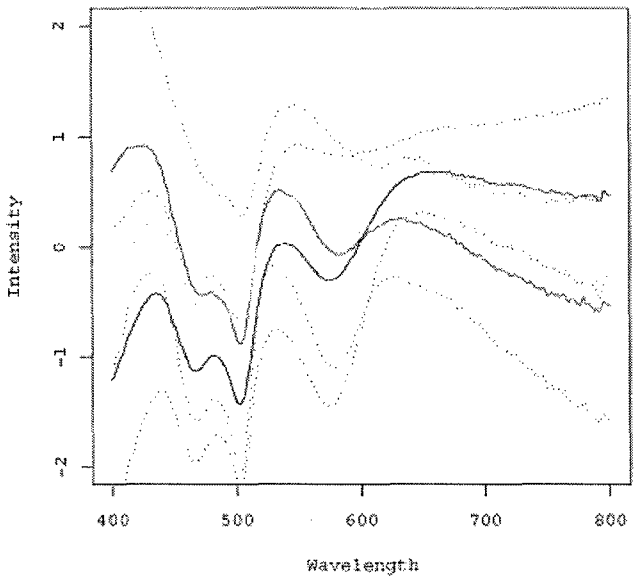

Figure 8. Mean spectra from normal and metastatic nodes. Blue line: normal nodes, red line: totally metastatic nodes, yellow line: partially metastatic nodes.

We have completed a re-analysis of this dataset recently. For this analysis a subset of 90% of normal and totally metastatic nodes was used as a training set to develop the discriminant algorithm. The accuracy of diagnosis of the remaining spectra (which was made up of the remaining normal and total metastatic nodes as well as the partial metastatic node) was calculated. The test and training sets were randomly re-sampled 30 times, and the results reported as an average of the 30 analyses. Our current algorithm differentiates total metastatic nodes from normal nodes with a sensitivity of 85% and a specificity of 87%. Detection of metastases in nodes involved with smaller amounts of tumour is with a sensitivity of 78% and specificity of 77%. A breakdown of the accuracy of diagnosis for different subclasses of nodes is given in table 1 below. It is important to note the due to the high percentage (90%) of normal and total metastatic nodes used in the training set to develop the algorithm, which are the spectra most likely to be correctly classified, the results here would underestimate the diagnostic accuracy likely to be achieved if tested prospectively.

Table 2.
Sensitivity of Diagnosis by Node Subclass

Total Metastases	88.3%
Partial Metastases	51.8%
Micro Metastases	39.1%
IHC Positive Nodes	0%
Specificity of Diagnosis	
Normal Nodes	88.4% (11.6% false positive)
Overall Accuracy	78.5% (Sensitivity 71.7%, Specificity 71.9%)

The poorer results with nodes that are only partly metastatic are almost certainly due to sampling errors. It is not surprising that if only a small percentage of the volume of the node is interrogated, then areas of cancer may be missed. More detailed optical sampling is required to improve sensitivity. There are 2 complementary approaches to this:

1. Increase the number of surfaces sampled (by cutting the node into more slices)
2. Perform more exhaustive ESS sampling of the cut surface of the node, by taking spectra from many more sites on each surface.

We have developed a prototype device that is able to perform multiple ESS measurements across the cut surface of the node. The device consists of a static standard ESS probe and a mobile X-Y stage, which is moved incrementally. We anticipate that this will enhance the ability to detect small metastatic deposits. Further, we think that developing an image of the scanned surface by plotting the results on a matrix may improve the specificity of diagnosis. A single spectrum suggestive of cancer might be given low weighting, but a cluster of positive spectra would be given more weight.

We have defined a relationship between measuring spectra with and without the scanning device, and performed scans of 13 nodes, which include normal nodes, partially metastatic nodes and completely metastatic nodes from both lobular and ductal carcinoma. Initial analysis of the spectra has proved encouraging, although much more data are required before conclusions can be drawn.

Specificity of diagnosis is the key to the value of this diagnostic technique because a false positive diagnosis of cancer could result in an unnecessary axillary lymph node dissection (axillary clearance). This would negate the main purpose of doing sentinel node biopsies, which is to reduce the morbidity of axillary dissection by limiting it to the sentinel node in patients who do not have cancer in the sentinel node.

We are taking 3 approaches to improve specificity:

1. Shifting the threshold for diagnosis: By moving the threshold canonical score, specificity can be improved at the expense of sensitivity.
2. Increasing the threshold number of spectra within a node required for a diagnosis of metastases.
3. By using 2-dimensional imaging – analysis of clustering of positive spectra, which may be a useful tool to improve specificity. We intend to explore this further with our 2-dimensional scanning studies.

We do not yet have results from these approaches.

The plan in the original statement of work was to also collect spectra from guided mammotome biopsies. Unfortunately, the institution's mammotome biopsy unit is not currently functional, due to the inability to recruit a technician to operate the device. All patients for mammotome biopsies are currently being referred to another unit. This aspect of the project has therefore had to be suspended until our own unit is functional.

Objective 3. Testing the ability of ESS to detect aneuploidy.

In recent years there has been much interest in developing molecular and cytogenetic markers of prognosis in breast cancer. Reliable markers of prognosis facilitate identification of patients at high risk of systemic metastases, to enable administration of adjuvant systemic therapy (chemotherapy).

Aneuploidy is one such potential marker of prognosis. Aneuploidy is abnormal DNA content (any variation from the normal diploid number of chromosomes). At certain wavelengths, the most significant contribution to the ESS spectra is scattering from cell organelles, particularly the nucleus. Alterations in chromatin content, as occurs in aneuploidy, give rise to localized changes in refractive index of subcellular components, which change the ESS spectra. Recent experiments by our collaborators, and others, have shown that ESS spectra can detect alterations in cellular chromosomal content ex-vivo, a phenonomen closely related to aneuploidy.

Much of the work on this objective so far has been technical work in the laboratory to set up and operate a Fairfield automated image cytometer, which was purchased by the Department of Histopathology for other projects. That department has also appointed a research pathology technician who will soon be able to process specimens routinely for this and other projects. Teething problems with methodology have been resolved, and 10 archived breast tumors have been analyzed, with satisfactory DNA histograms obtained.

To date tumors from 6 patients have been recruited to our ploidy study. For this, large tumors are divided into smaller blocks, so that so far we have 320 spectra from 32 separate fresh blocks of tumor, which will each have their ploidy status determined (the ploidy status is not necessarily the same throughout any one tumor). Ploidy studies are undertaken on formalin fixed, paraffin embedded tissue, so could be done on archived tissue. Consequently, we plan to take ESS spectra on both fresh and formalin fixed embedded tissue and compare the two. If we can detect aneuploidy using spectra from the fixed tissue, it would dramatically increase the number of tumors available for examination. Of course, our ultimate aim is to detect aneuploidy in vivo using ESS, as this has been shown to have important prognostic implications in breast carcinoma (as well as other cancers).(4-6)

The 2 dimensional scanning device described above has been used to obtain detailed ESS scans from 4 of these primary tumors both before and after formalin fixation, and this process will be repeated after paraffin embedding.

We do not yet have enough data to start trying to analyze the spectra to detect the aneuploidy.

Objective 4. To develop ductoscopy (nipple endoscopy) to detect intraductal lesions and determine their nature using ESS.

80% of breast cancers are ductal in origin. Engineering advances have resulted in the production of endoscopes with an external diameter less than 1mm. These micro-endoscopes may be introduced through the nipple to examine the breast ducts, and this procedure is called ductoscopy or nipple endoscopy.

The most promising roles for ductoscopy are:
1. The evaluation of symptomatic nipple discharge – which may be a presenting symptom of breast cancer(7)
2. Early detection of breast cancer – in theory, ductoscopes are capable of visualizing diseased areas as small as 0.2mm, many years before they can be detected with conventional imaging. In addition premalignant lesions (eg. atypical ductal hyperplasia) and ductal carcinoma-in-situ (DCIS) may be visualized(8).
3. Aiding margin determination in breast conserving surgery – by detecting intraductal spread of carcinoma(9).

A key problem is the inability to obtain histological samples for definitive diagnosis of lesions identified at ductoscopy. Our study is to explore the ability of elastic scattering spectroscopy to diagnose pathology seen at ductoscopy. Ductoscopy is carried out by first inserting a thin dilator (like a nylon suture) into a breast duct orifice in the nipple, followed by passing a tubular sheath over this. The dilator can then be removed and the rigid ductoscope passed through the sheath. Unfortunately, the ductoscope is too small to have a biopsy channel and it is not currently possible even to use biopsy forceps through the sheath alone. However, it is practical to pass an ESS probe through the sheath if the ductoscope is removed.

The study has 2 phases :
1. Ex-vivo study: On mastectomy specimens to develop the technique and prove the principle
2. In-vivo study: On patients with symptomatic nipple discharge or diagnosed breast carcinoma.

Hardware development:

An ESS optical biopsy probe with external dimensions of 0.8mm, which can be introduced through the ductoscopy sheath, has been produced by our collaborators in Boston, USA. This probe consists of 2 optical fibers with a diameter of 200 microns, with a centre-to-centre separation of 280 microns.

Ductoscopy Procedures :

There is undoubtedly a learning curve for performing ductoscopy. While other authors have reporting accessing up to 5 ducts per breast, to date we have not been able to access any more than 2 ducts for any one patient. The lead surgeon on the program, Mr. Keshtgar, has visited Professor William Dooley at the University of Oklahoma in June 2005. Professor Dooley is

an internationally acknowledged leader in the development of ductoscopy. The visit proved extremely valuable for developing technical skills. It is anticipated that incorporating the technical guidance from Prof. Dooley will improve both the number and length of ducts successfully examined and consequently increase the diagnostic yield of ductoscopy.

To date, we have performed 6 ex vivo ductoscopy procedures on the tissue removed from patients undergoing mastectomy for breast carcinoma (5 patients) or prophylactic mastectomy (1 patient) (Fig 7). We have successfully cannulated ducts in 5/6 (83%) of patients, though the number of ducts cannulated per patient has averaged less than 2. The newly developed fiber has been used to measure ESS spectra from normal ducts, DCIS and invasive cancer. We do not yet have enough spectra to perform an analysis of the ability of the system to detect cancer, but examples of spectra are given in the figure below. Preliminary examination of these spectra shows differences in hemoglobin absorption peaks (compatible with the avascular nature of DCIS) and differences in the spectra within the infra-red region, which may be useful for discrimination.

We have developed a method for obtaining conventional tissue biopsies to correlate with the ESS measurements. The ductoscopy light is used either to guide direct tissue biopsies or to mark the duct with a suture, so a biopsy can be taken when the ductoscopy procedure has been completed. (figure 8).

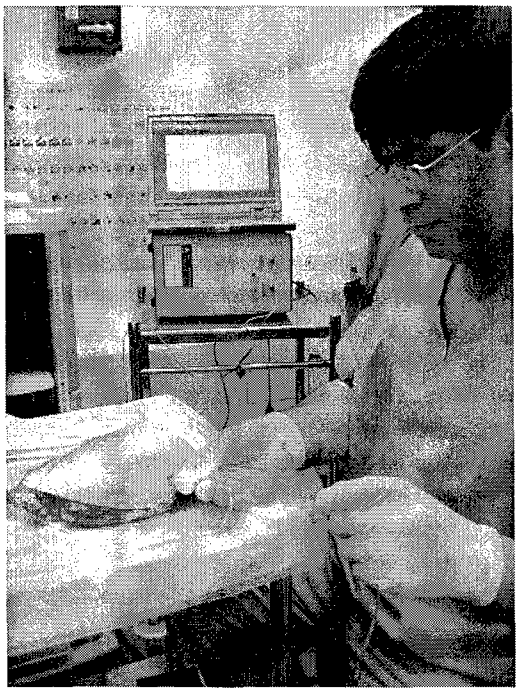

Fig 8. Ex vivo ductoscopy

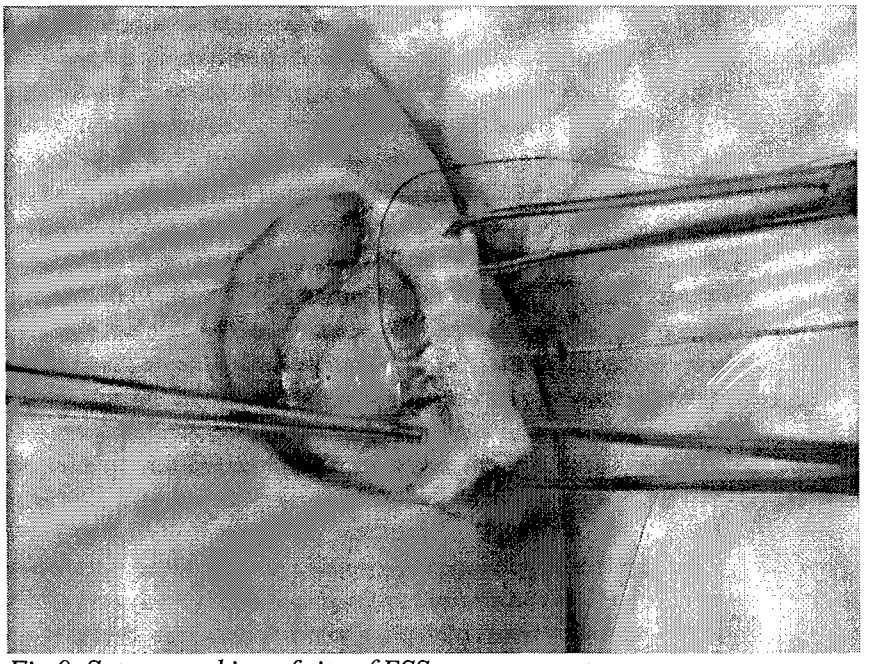

Fig 9. Suture marking of site of ESS measurement

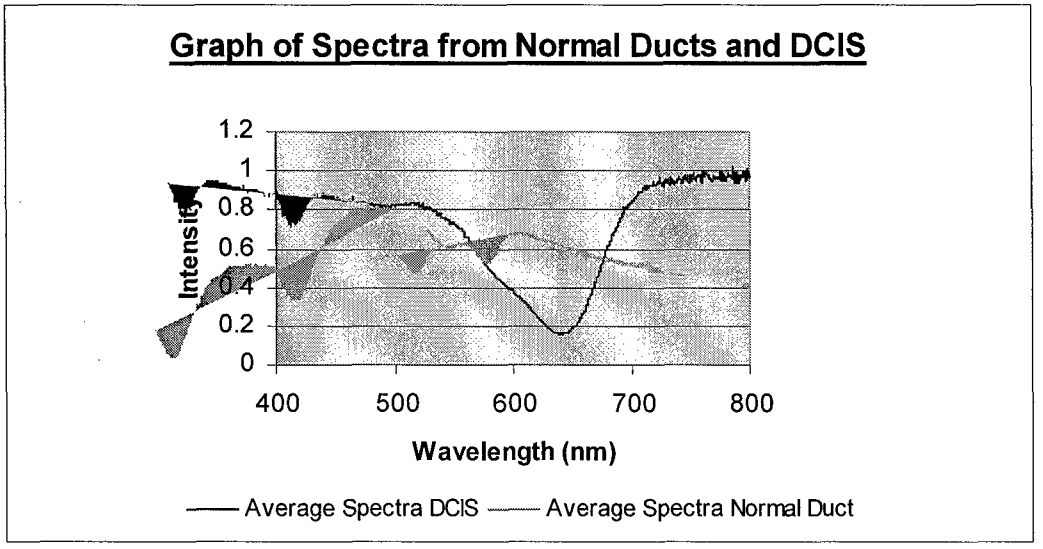

Fig 10. Examples of spectra obtained at ductoscopy from normal tissue and DCIS

The next step is to increase the number of ductoscope examinations on ex vivo tissue. If the results are good enough, we shall seek new IRB approval to undertake these measurements in vivo.

Key Research Accomplishments

1. We have demonstrated the ability of ESS to differentiate between cancer and normal breast tissue with high specificity.
2. We have demonstrated that ESS retains this ability to detect cancer, even when only a small percentage of the tissue volume interrogated by the light pulse is cancer.
3. We have expanded our database of ESS spectra from sentinel and axillary nodes with matched histology and are continually revising the algorithm for analysis of these spectra.
4. We have addressed the problem of sampling errors leading to cancer in sentinel nodes failing to be detected by building a prototype 2 dimensional scanning device.
5. We have developed an optical probe that is able to measure ESS spectra at ductoscopy and developed methodology for matching histology with these spectra. We are recruiting patients to this study.
6. We have optimized the methodology for DNA ploidy determination using image cytometry and are starting to build a database to correlate ESS spectra with image cytometry, looking for algorithms to detect aneuploidy from the spectra.

Reportable Outcomes

Presentations

1. 4th International Sentinel Node Conference, Santa Monica, California, USA, 3rd-6th December 2004 : Instantaneous detection of sentinel node metastases in breast cancer by "optical biopsy" using elastic scattering spectroscopy. D. Chicken, K Johnson, M Falzon, W. Waddington, P. Ell, S Bown, M Keshtgar.

2. 27th Annual San Antonio Breast Cancer Conference, San Antonio, Texas. USA, 8th-11th December 2004: Optical biopsy utilizing elastic scattering spectroscopy for the intraoperative determination of sentinel node status in breast carcinoma. Chicken DW, Johnson KS, Bown SG, Bigio IJ, Keshtgar MRS

3. British Medical Laser Association Autumn Meeting, 9th & 10th September 2004, London, UK. The application of light in the diagnosis and management of breast disease *MRS Keshtgar, DW Chicken*

4. *4th International Symposium on the Intraductal Approach to Breast Cancer, 11th-13th March 2005, Santa Barbara, California, USA.* Light for Lightning Diagnosis. MRS Keshtgar

5. *US Army Era of Hope Conference, June 8th-11th 2005, Philadelphia, Pennsylvania, USA.* Optical Biopsy for Real-time Diagnosis, Staging and Prognostication in Breast Cancer M Keshtgar, D Chicken, A Lee, G Briggs, K Johnson, B Clark, D Pickard, M Falzon, I Bigio & S Bown

6. European Conference on Biomedical Optics, Munich, Germany, 12th – 16th June 2005, Munich, Germany. Elastic Scattering Spectroscopy for Detection of Sentinel Lymph Node Metastases in Breast Carcinoma. DW Chicken, AC Lee, KS Johnson, B Clarke, M Falzon, IJ Bigio, SG Bown, MRS Keshtgar.

Published Abstracts

Optical biopsy utilising elastic scattering spectroscopy for intra-operative diagnosis of sentinel lymph node metastases. Chicken DW, Johnson KS, Falzon MR, Lee AC, Briggs G, Pickard D, Bigio IJ, Bown SG, Keshtgar MRS. JOURNAL OF CLINICAL ONCOLOGY 22 (14): 841 Suppl. S JUL 15 2004

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Published Papers

Elastic scattering spectroscopy for intraoperative determination of sentinel lymph node status in the breast. Johnson KS, Chicken DW, Pickard DCO, Lee AC, Briggs G, Falzon M, Bigio IJ, Keshtgar MR, Bown SG. JOURNAL OF BIOMEDICAL OPTICS 9 (6): 1122-1128 NOV-DEC 2004

Elastic Scattering Spectroscopy for Detection of Sentinel Lymph Node Metastases in Breast Carcinoma. DW Chicken, AC Lee, KS Johnson, B Clarke, M Falzon, IJ Bigio, SG Bown, MRS Keshtgar. SPIE PROCEEDINGS
JUNE 2005 (In Press)

Submission of Higher Degrees

The research fellow, Wayne Chicken will be submitting a doctoral thesis to the University of London based on the work performed under this grant.

Conclusions

Elastic scattering spectroscopy is a relatively simple and low cost technique, which has the potential to provide an immediate answer as to whether or not cancer is present in breast or lymph node tissue. As the spectra are analyzed by computer algorithm, no pathological interpretation is required, so the results are essentially operator independent. Our results so far have shown quite good sensitivity and specificity for detecting cancer, but for each objective, considerably more data are required before definitive conclusions can be reached.

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